ARS SPECIAL REPORT

Antibiotics for the Control of Vegetable Crop Diseases

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SUMMARY

Research has clearly indicated that antibiotics will control certain major plant diseases and in some cases eradicate infection of treated plants. These complex organic chemicals, which inhibit or destroy the growth of disease-causing organisms, have made phenomenal contributions to the fields of human medicine and animal nutrition.

Now pathologists have successfully directed the action of antibiotics against certain plant diseases. The success of antibiotics in this field is credited to their ability to enter into the plant where they protect against invasion of the disease-causing organism and stop the growth of organisms already in the plant, rather than serving simply as a surface protectant.

Commercial formulations of some antibiotics are available to vegetable growers, but they have not come into wide use. This report discusses these antibiotics, recently developed ones which show promise, and the specific diseases which each has controlled.

Information in this report was provided by the Horticultural Crops Research Branch, Agricultural Research Service

ANTIBIOTICS FOR THE CONTROL OF VEGETABLE CROP DISEASES

For many years the two most effective weapons against plant diseases have been the breeding of resistant varieties and the use of chemical sprays or dusts. Both methods have been effective against diseases caused by fungi, but neither has been very effective against bacterial diseases. Recently, however, scientists have found that some of the same antibiotics used in human medicine are also capable of destroying various organisms which cause disease in plants.

Antibiotics themselves are a recent discovery. But for millions of years soil microorganisms doubtless have been manufacturing these complex substances for use against their own microbe enemies. Just before World War II scientists isolated antibiotics and began using them against the microbes which cause various diseases of animals and plants. Little is known of the action of antibiotics except that they destroy or inhibit the growth of certain organisms. Penicillin is an outstanding example of this early work.

At first antibiotics were scarce and expensive and were used only to fight human ills. As the supply increased, they found a place in veterinary medicine, animal nutrition, and plant pathology. Only in the last 5 years has the use of antibiotics for the control of plant diseases begun to develop.

With the discovery that certain antibiotics would kill disease-causing bacteria in plants, came the discovery that they were systemic in their action. Unlike earlier fungicides and bacteriacides, which formed a protective coating over the plant's surface, most antibiotics are absorbed by the plant and move to its various parts. Once antibiotics have entered the plant, they are unaffected by rainfall and can move through the plant to protect internal tissues against the disease-causing organisms, or pathogen, and to destroy any organisms that have gained entrance. The degree of translocation, or movement through the plant, varies with the different antibiotics and with the plant treated, but all are systemic to some degree.

Many antibiotics have been tested by plant pathologists of the Agricultural Research Service of the U.S. Department of Agriculture, of State agricultural experiment stations, and of the pharmaceutical industry. Only a few have demonstrated their usefulness as specific plant-disease fighters. Of these few, streptomycin is the most effective in combating vegetable diseases.

STREPTOMYCIN

Streptomycin acts principally against disease-causing bacteria, but it is also effective in combating certain fungi. The bacteria causing halo blight of beans, bacterial spot of tomatoes, angular leaf spot of cucumbers, seed piece decay and blackleg of potatoes all can be controlled by streptomycin. In addition, the antibiotic controls the fungi causing downy mildew of lima beans, late blight of tomatoes, and downy mildew of cucurbits.

Streptomycin used for plant treatment is only partially purified, but it appears to be more effective for this use than the highly purified streptomycin of human medicine. At the present time only 4 known commercial streptomycin plant preparations--each containing different amounts of the antibiotic--are available. They are: Acco Streptomycin, Agristrep, Agri-mycin 100, all of which are dusts, and Phytomycin, which is a liquid.[1] Acco Streptomycin contains 45 percent streptomycin; Agristrep contains 37 percent; Agri-mycin 100 contains 15 percent; and Phytomycin contains 20 percent. Agri-mycin 100 contains, in addition, 1.5 percent of oxytetracycline (Terramycin) to prevent or retard the development of streptomycin-resistant strains of bacteria.

Reported here are the results of experiments in which streptomycin--in a pure form as well as in these commercial formulations--was successful in the control of specific vegetable diseases. No attempt is made to report all of the work done with this antibiotic on these diseases, but rather to present some typical results. Treatment and concentrations are not presented as recommendations but simply to provide information for those interested in the potential of antibiotics for the treatment of vegetable crop diseases.

Concentrations are referred to as parts per million (p.p.m.) rather than pounds per gallon, since only minute amounts of the antibiotics are required. A concentration of 100 p.p.m. refers to 100 parts of the active antibiotic in 1 million parts of water. This is the equivalent of 38 grams (about 1.34 ounces) dissolved in 100 gallons of water. Since the commercial formulations contain inert ingredients, a greater amount of these would have to be mixed with 100 gallons to obtain a concentration of 100 parts of the active antibiotic per million parts of water.

DISEASES OF BEANS

Halo Blight

Halo blight appears wherever rain falls frequently during the growing season. Only a few small areas of the Far West are free from the disease. When it is widespread it can cause serious losses.

During periods of high rainfall the causal bacterium, *Pseudomonas phaseolicola*, causes water-soaked spots to show on the leaves, stems and pods. Halo blight makes a halo-like zone of greenish-yellow tissue around each water-soaked spot during cool weather. Leaves of newly infected plants are yellow. As the infection advances, leaves turn brown and fall off.

Seed treatment is of doubtful value in the control of halo blight. Spraying or dusting the plants with fungicides has not proved to be a practical control measure.

Researchers at the United States Department of Agriculture's Agricultural Research Center, Beltsville, Md., not only controlled halo blight with the antibiotic streptomycin but largely eradicated it from the treated plants. It was the first time the use of an antibiotic controlled a bacterial disease in a field test.

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[1] Mention of these products does not constitute an endorsement by the U.S. Department of Agriculture over other products or a guaranty or warranty of the standard of these products.

First experiments were undertaken in the greenhouse, where minute amounts of streptomycin were applied to the stems of bean plants before inoculation of the leaves with the causal bacterium. The stems absorbed the antibiotic and carried it up to the leaves in sufficient amounts to protect them from infection.

In subsequent field tests the disease was effectively controlled with varying amounts of streptomycin, and in 1954 it was largely eradicated from treated plants with an Agri-mycin spray. In plots treated 3 times at weekly intervals with spray containing 200 p.p.m. of streptomycin, only 0.8 percent of the plants were infected, as compared with 19 percent of the untreated plants. The first spray was applied when the first signs of halo blight appeared. Plots treated similarly with an Agri-mycin spray containing 500 p.p.m. of streptomycin, beginning when the symptoms were more advanced, showed no active infections shortly after treatment. In the untreated plots, 26.5 percent of the plants were infected.

Common Blight

Caused by the bacterium, *Xanthomonas phaseoli*, common blight differs little from halo blight in observable symptoms.

In greenhouse tests at the Agricultural Research Center, streptomycin, applied at a concentration of 1,000 p.p.m., protected bean plants from common blight. Field plots that were thoroughly sprayed 3 times with the same concentration developed some diseased plants late in the season.

Downy Mildew

Downy mildew of lima beans is caused by a fungus, *Phytophthora phaseoli*. It has caused considerable damage in some of the Middle Atlantic and North Atlantic States, but it is of minor importance elsewhere.

The disease is recognized by the white cottony growth that forms in large, irregular patches on the pods. When young pods are attacked and when older ones are covered with the fungus growth, they shrivel, wilt, and die.

Clean cultivation, crop rotation, and the planting of seed from an uninfested crop are recommended. Dusting with a copper dust at weekly intervals during periods of wet weather has been helpful in control of the fungus but does not give full protection.

In ARS greenhouse tests excellent control of downy mildew was obtained with commercial streptomycin formulations. Almost complete protection resulted when lima bean seedlings were sprayed with these formulations at concentrations of 100 p.p.m. of streptomycin and were inoculated 12 to 24 hours later with spores of the downy mildew fungus. Almost complete control also resulted from an Agri-mycin spray containing only 50 p.p.m. of streptomycin and the same amount of a neutral copper fungicide.

A dust containing 1,000 p.p.m. of streptomycin gave the same protection against downy mildew as sprays containing 100 p.p.m. On an acre basis, dust would be cheaper per application than the spray, despite the higher concentration. To be effective, however, the dust has to be applied when moisture is on the plants, since the antibiotic is absorbed by the leaves or stems only if moisture is present.

In field tests, lima bean plots sprayed with Agri-mycin containing 50 p.p.m. of streptomycin and the same amount of copper showed only about 6 percent of the pods infected, compared with 30 percent in the untreated plots. ARS scientists believe that a slightly higher antibiotic concentration and better plant coverage would have resulted in even less infection in the treated plots.

DISEASES OF TOMATOES

Late Blight

Late blight of tomatoes is caused by the fungus, Phytophthora infestans, which also causes late blight in potatoes. Prevalent during wet weather, it may appear in most of the commercial tomato-growing sections of the United States. Increased use of fungicides in recent years has greatly reduced loss from this tomato blight.

Agristrep, Agri-mycin, and Phytomycin sprays effectively protected tomato seedlings from late blight in greenhouse tests at the Agricultural Research Center. Eight-inch tomato seedlings sprayed with any of these commercial formulations at concentrations of 100 p.p.m. of streptomycin and inoculated with the tomato blight organisms 24 hours later remained almost totally free of the disease. Untreated plants were severely infected. Later inoculations without further treatment might well have infected the plants.

Bacterial Spot

One of the common diseases of tomatoes is bacterial spot, caused by the bacterium, Xanthomonas vesicatoria. It is most prevalent in the Middle Atlantic, South Atlantic, and Central States. The disease attacks both leaves and fruit, and results in substantial yield reductions. Various fungicides are used to combat it.

Control of bacterial spot with streptomycin in tomato plant beds and in commercial fields has been reported by the Florida Agricultural Experiment Station. In beds treated with an Agri-mycin spray containing 200 p.p.m. of streptomycin, 95 percent of the plants were suitable for transplanting as compared with only 27 percent of those in the untreated beds. Five sprays were applied at intervals of 2 to 4 days.

In tests with mature infected plants, which were sprayed when they were setting fruit, treated plots gave 42 percent greater yield, 28 percent greater average fruit size, and 11 percent greater number of fruits than untreated plots. Other tests, which included 7 applications, at 4 to 5 day intervals, of Agri-mycin at a concentration of 200 p.p.m. of streptomycin, and begun when the plants were 4 inches high, resulted in fewer infected plants and increased yield over untreated plots.

DISEASES OF PEPPERS

Bacterial Spot

Peppers are attacked by the same organism causing bacterial spot of tomatoes. At the Delaware Agricultural Experiment Station, 3 spray applications of streptomycin at a concentration of 500 p.p.m. eradicated the

disease in mature pepper plants. At the Florida Agricultural Experiment Station 5 applications at 200 p.p.m. effectively controlled the disease. Good results were also obtained in Florida when 2 to 4 pounds of a neutral copper fungicide in 100 gallons of water was combined with only 100 to 200 p.p.m. of streptomycin.

DISEASES OF CUCURBITS

Downy Mildew

Downy mildew is a destructive disease of cucumber, as well as muskmelon and watermelon, in the Eastern and Southern States. It is usually less damaging in the North Central States and rarely occurs in the Southwest. The causative fungus, *Pseudoperonospora cubensis*, also attacks pumpkin and squash.

The disease first appears as a gray-tinged spore mass on older leaves. It causes small, angular, yellowish spots, which later increase in number and size. Severely infected leaves turn yellow, then brown, and finally shrivel. Maturing fruits or infected plants fail to color properly, are tasteless, and usually are sunburned.

Various fungicidal sprays and dusts have proved useful in the past, but effective control with streptomycin has recently been reported. The Florida Agricultural Experiment Station used 7 applications of Agri-mycin at a concentration of 200 p.p.m. of streptomycin to achieve control on cucumbers. Plants were sprayed at 4 to 5 day intervals. The same number of applications of streptomycin (100 p.p.m.) and tribasic copper (2 to 4 pounds per 100 gallons of water) proved equally effective.

Angular Leaf Spot

Angular leaf spot is serious only in cucumbers. The causal organism, *Pseudomonas lachrymans*, forms water-soaked spots on leaves and fruits. The spots become angular, turn gray to tan in color, and form an exudate on the lower surface of the leaf. Many of the infected spots loosen and fall out. Infected fruits show a brown, firm rot which ruins salability. Chances of seedling infection can be reduced by soaking the seed in mercuric chloride, but the treatment may not prevent an occurrence of the disease if conditions favor its development.

Field-grown cucumber plants at the Agricultural Research Center were protected from angular leaf spot by spraying with either Agri-mycin or Agristrep at a concentration of 400 p.p.m. of streptomycin the day before inoculation. Infection was further reduced to less than 1 infected leaf per plant by additional sprayings on the fifth, tenth, and fifteenth day after inoculation.

DISEASES OF POTATOES

Seed-Piece Decay and Blackleg

Decay of potato seed pieces is caused by various bacteria, including *Erwinia atroseptica* and *Pseudomonas fluorescens*. These organisms frequently cause heavy losses in field stands and of potatoes in storage.

E. atroseptica also causes blackleg of potato plants which is prevalent during wet seasons. When the plants show rigid, rolled leaves that yellow, wilt, and die, blackleg can be suspected. If tubers are produced they show a soft rot which may involve the entire potato or cause a black, rotted center. In either case salability is impaired or destroyed.

The Maine Agricultural Experiment Station found that an instant dip of potato seed pieces in an Agri-mycin solution containing 100 p.p.m. of streptomycin practically eliminated seed-piece decay. On the other hand, 80 to 100 percent of the untreated seed pieces decayed. Treatment increased emergence in the field, reduced the percentage of blackleg, improved the color, size, and vigor of the plants, and increased yield by 19 to 72 bushels an acre.

When cut seed pieces were treated in Phytomycin solutions containing 50 to 100 p.p.m. of streptomycin, seed-piece decay was reduced and plant height, number of stems per hill, number of flower clusters per plant, and yield rate were all increased.

DISEASES OF CELERY

Bacterial Blight

Bacterial blight of celery, caused by Pseudomonas apii, is an important disease in celery seedbeds in the Everglades section of Florida. The bacterium causes small, irregularly shaped spots on the leaflets which change from yellow to brown and materially lower celery grade.

A high degree of control can be obtained with 5 applications of copper A (4 pounds in 100 gallons of water), but the disease shows a tendency to build up.

The Florida Agricultural Experiment Station has reported that the same number of treatments with Agri-mycin, at concentrations ranging from 300 to 600 p.p.m. of streptomycin, was more effective than copper A, because the severity of the disease diminished. A combination of the two materials gave better control than either one separately, but not significantly better than Agri-mycin.

NEW ANTIBIOTICS

Research is continuing to isolate new antibiotics, such as Oligomycin, Griseofulvin, Filipin, Anisomycin, Mycostatin, Rimocydin,[2] and F-17. None of these are commercially available.

The first six of these seven antibiotics have recently been tested by Agricultural Research Service scientists to determine their effectiveness against fungi causing rust and anthracnose of snap and dry beans and those causing downy mildew and stem anthracnose of lima beans. Bean plants were sprayed with water solutions or suspensions of the six antibiotics, and 24 hours later were inoculated with spore suspensions of the disease-causing fungi. They were then placed in a fog chamber to provide humidity favorable to infection. After 24 hours the plants were removed and held in an open greenhouse for observation.

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Table 1.--Experience with streptomycin[1] in the control of vegetable crop diseases

Disease	Causal organism	Treatment	Number of treatments	Interval between treatments	Time of application	Concentration[2]	Effective under	
							Greenhouse conditions	Field conditions
				Days		P.p.m.		
Halo blight of beans	Bacterium	Spray	3	7	Before and after infection	200 to 500	Yes	Yes
Common blight of beans	do.	do.	1 to 3	7	Before infection	1,000	do.	No
Downy mildew of lima beans	Fungus	Dust or spray	1	---	do.	[3] 50 to 100 [4] 1,000	do.	Do.
Late blight of tomatoes	do.	Spray	1	---	do.	100	do.	Do.
Bacterial spot of tomatoes	Bacterium	do.	5 to 7	2 to 5	Seedlings: before infection Plants: at first symptoms	200	No	Yes
Bacterial spot of peppers	do.	do.	[5] 3 to [6] 5	7	Before and after infection	200 to 500	do.	Do.
Downy mildew of cucurbits	Fungus	do.	7	4 to 5	At first symptoms	[7] 100 to 200	do.	Do.
Angular leaf spot of cucumbers	Bacterium	do.	3	5	Before and after infection	400	Yes	Do.
Seed-piece decay of potatoes	do.	Dip	1	---	Before planting	50 to 100	do.	Do.
Blackleg of potatoes	do.	do.	1	---	do.	50 to 100	do.	Do.
Bacterial blight of celery	do.	Spray	5	Not reported	Before infection	300 to 600	No	Do.

[1] One commercial formulation of streptomycin, Agri-mycin 100, also contained oxytetracycline (Terramycin).

[2] Parts of active streptomycin per million parts of water.

[3] As a spray.

[4] As a dust.

[5] At 500 p.p.m.

[6] At 200 p.p.m.

[7] Plus 2 to 4 pounds of copper per 100 gallons of water.

Tests with the seventh antibiotic, F-17, have been run to determine its effectiveness against rust in beans and wheat.

A brief description of these new antibiotics and a statement of their performance in the ARS tests follows.

Oligomycin

Oligomycin was isolated by University of Wisconsin scientists. In laboratory tests at the Wisconsin Agricultural Experiment Station, 19 plant-disease organisms proved sensitive to it. In greenhouse tests there, it was first added to soil in which alfalfa seed was planted and later applied to seedlings in water. The antibiotic afforded complete protection against a species of Pythium, a type of fungus which causes damping-off in plants of several commercial crops. This genus of fungi constitutes one of the most destructive groups of plant pathogens that are generally uncontrolled by the best methods known today.

Results of ARS Bean Tests

Oligomycin was the most effective of the six antibiotics included in the comparative tests against 4 diseases of beans. It was toxic to all four disease organisms when applied to the plants at a concentration of 100 p.p.m.

Griseofulvin

Griseofulvin was isolated in England and is produced by a species of Penicillium which is related to the organism from which penicillin is obtained. The discoverers of this antibiotic report that a large number of fungi on artificial media proved sensitive to it, and that it protected foliage against powdery mildew of barley, gray mold of lettuce, and Alternaria or early blight of tomatoes. It has also been reported that it is translocated within plants, that it may be more effective through root than leaf absorption, and that it persists in the tissue of tomato leaves.

Results of ARS Bean Tests

Griseofulvin protected beans from rust but was ineffective against the other 3 diseases. It is believed, however, that use of a different solvent to obtain a water suspension of this antibiotic would have resulted in greatly increased effectiveness.

Filipin

University of Illinois scientists, who isolated Filipin, report that it has demonstrated protection against some seed-rotting fungi, and that dust-seed treatments completely protected cucumber and spinach seedlings against pre-emergence damping-off caused by Rhizoctonia, another destructive species of plant-disease organism. Spraying young tomato and bean plants at a concentration of 1,000 p.p.m. of this antibiotic was reported to have caused no damage.

Results of ARS Bean Tests

Filipin protected lima beans from downy mildew, and partially protected beans against anthracnose, but the concentrations used were exceptionally high.

Anisomycin, Mycostatin, and Rimocydin

These three antibiotics, like Oligomycin and Filipin, are produced by Streptomyces, the genus of molds that provides streptomycin. Anisomycin, Mycostatin, and Rimocydin are products of American pharmaceutical companies and show promise as anti-fungal agents.

Results of ARS Bean Tests

Anisomycin, at 50 p.p.m., protected beans from rust and lima beans from downy mildew infection. At 100 p.p.m., this antibiotic eradicated rust from plants that had been infected as long as 72 hours before the antibiotic treatment. Of the 6 new drugs in this test only Anisomycin demonstrated eradicative powers at the dosages used.

Mycostatin sprays protected beans from anthracnose, gave partial protection to beans against infection by rust, and partial protection to lima beans against downy mildew.

Rimocydin gave partial protection to lima beans against anthracnose.

F-17

F-17 is a product of the research program of the ARS Northern Utilization Research Branch, Peoria, Ill. Although all of the plant disease-control antibiotics used today are by-products of research in the field of human medicine, ARS scientists believed that antibiotics of great importance could be developed specifically for plant-disease control. Accordingly, some 500 strains of the Streptomyces group of antibiotic-producing microorganisms were selected for primary screening in 1953. Tests with over a dozen different disease-causing bacteria or fungi cut the field to 200. Further screening reduced the number to 52 microorganisms. Ten of these were selected for the production of antibiotics for immediate greenhouse testing.

F-17 is the final result of this basic research. It is the product of the S. cinnamoneous strain, and is made up of three-- and possibly four-- separate antibiotics. This crude mixture has proved effective against fungi on artifical media and on plants in the greenhouse for the control of rust in both beans and wheat.

ECONOMIC ASPECTS

The current cost of antibiotics restricts their use to those crops with a high value per acre or where control requires only small quantities of spray materials. Lower cost may come about in either of two ways: First, as the use of antibiotics for plant disease control widens and volume increases, mass production of agricultural antibiotics may bring about reduced prices. Secondly, new and less highly purified antibiotics, selected specifically for plant disease control, may prove effective at low concentrations.

Considerable attention is being directed toward the use of antibiotics as seed treatments. In such case only small quantities would be necessary and application costs would be nominal. The ideal seed treatment would be inexpensive to apply, would not be inactivated or destroyed in the soil,

would not destroy beneficial microorganisms, and would not be toxic to crop plants. In addition it would be readily absorbed by the roots, be translocated in the plant, and be effective against a large number of disease-causing organisms.

Oligomycin is one of the new antibiotics that may approach this ideal. University of Wisconsin scientists report that it is not inactivated or destroyed in the soil, does not destroy beneficial soil organisms, and is not toxic to plants at concentrations as high as 3,000 p.p.m. In addition, the antibiotic is absorbed by the roots and can be later detected in the stems. As mentioned earlier 19 plant-disease-producing organisms proved sensitive to it in the laboratory.

As promising as these new antibiotics are, the search for new and more effective ones is continuing. Past research has demonstrated the effectiveness of antibiotics in the destruction of pathogens and their ability to enter into the plant to control the disease-causing organism. If continuing research can also bring about reduced costs, antibiotics may soon provide the same ''miracle'' protection to crop plants that they are providing the human population.

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